Notes ichtyologiques / *Ichthyological notes*

Intraspecific chromosome analysis in *Rhamdia quelen* (Siluriformes, Heptapteridae)

by

Vivian Patricia Oliveira de MORAES, Juliana de Souza CARNEIRO & Ana Lúcia DIAS* (1)

RÉSUMÉ. - Analyse chromosomique intra-spécifique chez *Rhamdia quelen* (Siluriformes, Heptapteridae).

Nous avons étudié les chromosomes de *Rhamdia quelen* provenant de plusieurs sites du Brésil. Tous les spécimens présentent 2n = 58 avec 36m + 16 sm + 6st et FN = 116 avec des chromosomes métacentriques de taille moyenne. Les spécimens provenant des rivières Água dos Patos, Água das Pedras et d'une station de pisciculture (FUNPIVI) ont montré une constriction secondaire dans le bras court de la paire 28 (st), correspondant avec la NOR, tandis que dans la population de la rivière Taquari, la NOR a été observée dans la région terminale du bras court de la paire 23 (sm), où les chromosomes homologues présentaient un hétéromorphisme pour cette région. Les NORs correspondent avec une bande C-positive et fluorescente après coloration à la CMA3. L'hybridation *in situ*, d'une sonde ADNr 28S, a été réalisée uniquement pour la population de la pisciculture, confirmant la présence des cistrons ribosomaux dans une paire de chromosomes. Le patron de distribution de l'hétérochromatine a été similaire dans toutes les populations, étant limité à une faible coloration dans les régions terminales des chromosomes, ainsi que dans les régions portant les NORs. Les échantillons de *Rhamdia quelen* examinés dans cette étude ont démontré une similitude caryotypique entre eux, tant pour les spécimens d'une station de pisciculture que pour ceux du milieu naturel.

Keywords. - Heptapteridae - *Rhamdia quelen* - Cytogenetics - Populations - AgNORs - CMA$_3$ - FISH.

In most populations of *Rhamdia,* previous cytogenetic studies have revealed a diploid number of 58 and the data show a wide variation of the karyotype macrostructure, with differences in the karyotypic formulae of the various populations (Garcia *et al*., 2003). In *Rhamdia,* the nucleolar organizer region (NOR) is generally located, in the terminal region of the short arm of a submetacentric or on a subtelocentric chromosome (Garcia *et al*., 2010). The heterochromatin has been found in small quantities, basically distributed in the terminal regions of some chromosomes. In *R. quelen* (Garcia *et al*., 2010), the location of major ribosomal genes has been directly visualized by fluorescence *in situ* hybridization (FISH), mainly with 18S rDNA probes.

The aim of the present study was to perform an intraspecific analysis of specimens of *R. quelen* from four locations using various chromosome banding techniques, to expand our knowledge of the karyotype in this group of fishes.

MATERIAL AND METHODS

A total of 25 specimens of *Rhamdia quelen* from four localities were studied: 9 specimens (3 males, 5 females and 1 sex unknown - MZUEL 5385) collected in Água dos Patos River (Iepê, São Paulo State), 5 specimens (1 male and 4 females - MZUEL 5384) of Água das Pedras River (Londrina, Paraná State), 7 specimens (3 males, 3 females and 1 sex unknown - MZUEL 3996) from Taquari River (Jataizinho, Paraná State) and 4 specimens from the fish farm station named FUNPIVI (Fundação de Piscicultura do Vale do Itajaí), located in Timbó, Santa Catarina State. Chromosome preparations were obtained from kidney cells by direct method described for Bertollo *et al*. (1978) for the populations of Água das Pedras, Água dos Patos and Taquari rivers. For the specimens from the fish farm station and also Água das Pedras River, the chromosomes were obtained through lymphocyte culture, following the method by Fenocchio and Bertollo (1988). The chromosomes were classified in metacentrics (m), submetacentrics (sm) and subtelocentrics (st), being all considered with two arms to calculate the fundamental

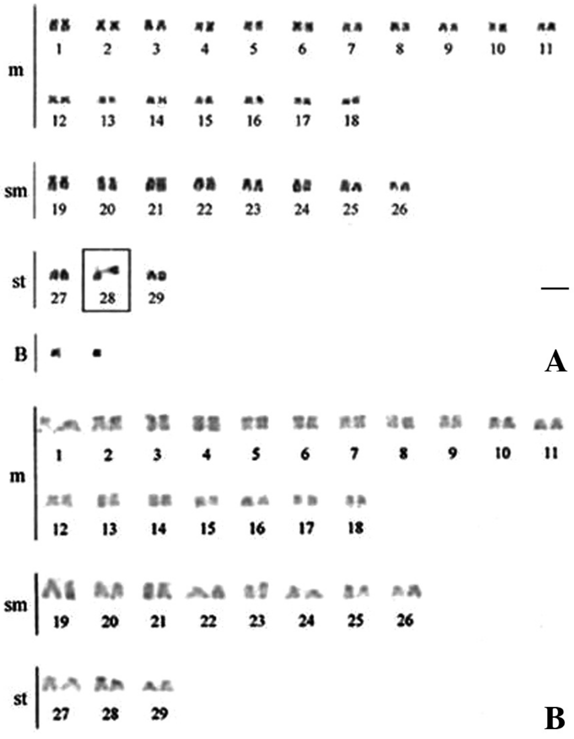

Figure 1. - Karyotype of *Rhamdia quelen*, representative from all populations. **A**: Giemsa staining. m = metacentric, sm = submetacentric, st = subtelocentric, B = B chromosome. In the box the pair 28 with secondary constriction; **B**: C banding. Scale bar = 10 μm.

---

(1) Departamento de Biologia Geral, CCB, Universidade Estadual de Londrina, Caixa Postal 6001, CEP 86051-970, Londrina, PR, Brasil. [vipamoraes@yahoo.com.br] [jucarneiro81@yahoo.com.br]

* Corresponding author [anadias@uel.br]

Cybium 2010, 34(4): 397-398.

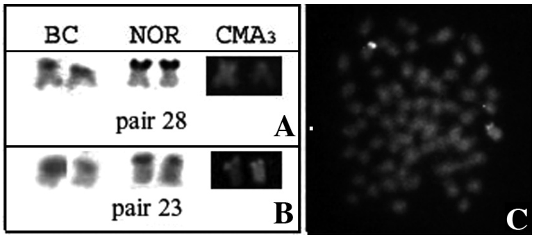

Figure 2. - **A**: Pair 28 from Água dos Patos River, Água das Pedras River and fish farm station with C banding, NOR and CMA$_3$; **B**: Pair 23 from Taquari River with C banding, NOR and CMA$_3$; **C**: FISH with 18S rDNA probe from fish farm statio

number (FN). NOR silver staining and C-banding were performed according to Howell and Black (1980) and Sumner (1972), respectively, and chromomycin A$_3$ (CMA$_3$) staining following Schmid (1980). A rDNA 18S probe was used for fluorescence *in situ* hybridization (FISH), according to Swarça *et al.* (2001).

RESULTS AND DISCUSSION

The specimens of *Rhamdia quelen* showed a diploid number of 58 with 36m + 16sm + 6st and FN = 116 (Fig. 1A). The 2n = 58 is a conserved characteristic in the genus *Rhamdia*; however, the constitution of 36m + 16sm + 6st was only described in *R. quelen* from Bodoquena Plateau (Moraes *et al.*, 2007) and results are different from all others (Garcia *et al.*, 2010), although it maintains a predominance of metacentric and submetacentric chromosomes and a fundamental number higher than 100, typical of the genus. Such differences in the karyotype formula can be attributed to chromosomal rearrangements that occurred in these populations over the years, or even due to difference in measurements of the chromosomal arms, according to various degree of chromosomal condensation. B chromosomes of the medium metacentric type were found in all populations, including the specimens from the fish farm station, but their number and frequency in each varied greatly, as related by Moraes *et al.* (2009).

C-banding produced weak staining of the terminal regions of almost all the chromosomes, where in some of them this staining was seen in both arms (Fig. 1B). The secondary constriction in pair 28 of the specimens from Água dos Patos, Água das Pedras and fish farm station was coincident with the NORs and appeared heterochromatic (Fig. 2A), as well as the terminal region of the short arm of pair 23 (sm) from the Taquari River population, in which heteromorphism was observed among the homologous chromosomes (Fig. 2B). The *in situ* hybridization confirmed a single location of rDNA on chromosome pair 28 in the fish farm station specimens (Fig. 2C). The morphological difference among the NOR-bearing chromosomes, in the populations and/or species of *Rhamdia*, as seen in this study, can be due to the occurrence of chromosomal rearrangements, such as inversions and/or translocations.

CMA$_3$ chromosomal staining showed fluorescent signals coincident with the NORs in *R. quelen* (Fig. 2A, 2B), indicating that these regions are flanked by GC-rich heterochromatin.

The distribution of only weak heterochromatic staining in the terminal regions in both chromosome arms was described by Fenocchio and Bertollo (1990) in *R. hilarii* and by Garcia *et al.* (2003) in *Rhamdia* sp., and therefore *R. quelen* is the first record of strong heterochromatic blocks at both chromosomal telomeres, which can be considered as a discriminative character in this group of fish.

In summary, the specimens of *R. quelen* from the four localities examined in this study demonstrated consistent karyotypic features in terms of diploid number, karyotype formulae, patterns of distribution and composition of heterochromatin, NOR location and the presence of B chromosomes. The only evident polymorphism was found in the NOR. According to present analysis and the literature for this genus, the karyotype evolution process in this group of fish is rather conservative, although the possibility of chromosomal rearrangements among some populations and/or species of *Rhamdia* cannot be discounted.

Acknowledgments. - The authors are grateful to Fundação Araucaria for financial support. We are also thankful to Dr. Albert Leyva for his help in the preparation of the manuscript.

REFERENCES

BERTOLLO L.A.C., TAKAHASHI C.S. & MOREIRA-FILHO O., 1978. - Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Braz. J. Genet.*, 1: 103-120.

FENOCCHIO A.S. & BERTOLLO L.A.C., 1988. - A simple method for fresh-water fish lymphocyte culture. *Braz. J. Genet.,* 11: 847-852.

FENOCCHIO A.S. & BERTOLLO L.A.C., 1990. - Supernumerary chromosomes in *Rhamdia hilarii* population (Pisces, Pimelodidae). *Genetica,* 81: 193-198.

GARCIA C., OLIVEIRA C. & ALMEIDA-TOLEDO L.F., 2010. - Karyotypic evolution trends in *Rhamdia quelen* (Siluriformes, Heptapteridae) with considerations about the origin and differentiation of its supernumerary chromosomes. *Genet. Mol. Res.*, 9: 365-384.

HOWELL W.M. & BLACK D.A., 1980. - Controlled silver staining of nucleus organizer regions with a protective colloidal developer: A 1-step method. *Experientia*, 36: 1014-1015.

MORAES V.P.O., CEREALI S.S., FROEHLICH O. & DIAS A.L., 2007. - Citogenetic caracterization of *Rhamdia quelen* (Siluriformes, Heptapteridae) from Bodoquena Plateau/MS. *Genet. Mol. Res.*, 6: 627-633.

MORAES V.P.O., CARNEIRO J.S. & DIAS A.L., 2009. - B chromosomes in four different populations of *Rhamdia quelen* (Siluriformes, Heptapteridae): a comparative study of the frequency and distribution. *Folia Biol.*, 57: 165-169.

SCHMID M., 1980. - Chromosome banding in Amphibia: IV. Differentiation of G-C and A-T rich chromosome regions in Anura. *Chromosoma*, 77: 83-103.

SUMNER A.T., 1972. - A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.*, 75:304-306.

SWARÇA A.C., GIULIANO-CAETANO L., VANZELA A.L.L. & DIAS A.L., 2001. - Heteromorphism of rDNA size in *Pinirampus pirinampu* (Pisces: Pimelodidae) detected by *in situ* hybridization. *Cytologia*, 66: 275-278.

Reçu le 22 septembre 2009.
Accepté pour publication le 20 août 2010.